May 23, 2023 Version 1

Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons V.1

Cell reports

DOI
dx.doi.org/10.17504/protocols.io.e6nvwj54dlmk/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwj54dlmk/v1

External link: https://doi.org/10.1016/j.celrep.2023.112448

Protocol Citation: Dan Dou, C. Alexander Boecker, Erika L.F. Holzbaur 2023. Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwj54dlmk/v1

Manuscript citation:
Pantazis, C.B., Yang, A., Lara, E., McDonough, J.A., Blauwendraat, C., Peng, L., Oguro, H., Zou, J., Sebesta, D., Pratt, G., et al. (2022). A reference induced pluripotent stem cell line for large-scale collaborative studies. BioRxiv 2021.12.15.472643.

Protocol status: Working
We use this protocol and it's working

Created: October 12, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 71238

Keywords: iPSC, Differentiation, iNeuron, Piggybac, NGN2, ASAPCRN, inducible ngn2 in human ipsc, glutamatergic neuron, stable expression of ngn2, employing piggybac transfection, ineuron differentiation from human ipsc, excitatory glutamatergic neuron, piggybac transfection, inducible ngn2, ngn2, stable integration of ngn2, mediated stable expression, piggybac, doxycycline, neuron, ineuron differentiation

Funders Acknowledgements:
ASAP
Grant ID: ASAP-000350

Abstract

We adapted a previously-described method (Pantazis et al., 2022) for employing Piggybac transfection to stably express doxycycline-inducible NGN2 in human iPSCs. After stable integration of NGN2, proceed to differentiate iPSCs using protocol “iNeuron differentiation from human iPSCs.”

Attachments

549-1145.pdf
106KB

Guidelines

Citations:

- Pantazis, C.B., Yang, A., Lara, E., McDonough, J.A., Blauwendraat, C., Peng, L., Oguro, H., Zou, J., Sebesta, D., Pratt, G., et al. (2022). A reference induced pluripotent stem cell line for large-scale collaborative studies. BioRxiv 2021.12.15.472643.

Materials

Materials

- 10 cm cell culture dish
- 6-well cell culture dish
- Cryovials

Reagents

- Growth Factor Reduced (GFR) Matrigel® **Corning** **Catalog #**354230
- Essential 8™ Medium **Gibco - Thermo Fisher Scientific** **Catalog #**A1517001
- Accutase® solution **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A6964
- Y-27632 2HCl **Selleckchem** **Catalog #**S1049
- Opti-MEM™ I Reduced Serum Medium **Thermo Fisher** **Catalog #**31985070
- Lipofectamine™ Stem Transfection Reagent **Thermo Fisher Scientific** **Catalog #**STEM00008
- PB-TO-hNGN2 **addgene** **Catalog #**172115
- piggyBac™ transposase vector (Transposagen)
- KnockOut™ Serum Replacement **Thermo Fisher** **Catalog #**10828010
- DMSO (CATALOG)

Troubleshooting

Safety warnings

Wear proper PPE when transferring cryovials to liquid N2.

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3d 6h

1 Culture iPSCs in a 10 cm dish coated with Growth Factor Reduced Matrigel (Corning) and feed daily with Essential 8 media (ThermoFisher).

2 Passage iPSCs with warm Accutase into Essential 8 media with [M] 10 micromolar (µM) ROCK inhibitor. Plate 800,000 iPSCs into one Matrigel-coated well of a 6-well plate.

3 3 - 6 hours after plating, cells should be healthy and attached. Perform transfection using Lipofectamine Stem and a 2:1 ratio of donor plasmid to transposase:

	A	B
	OptiMEM	200 µL
	PB-TO-hNGN2-puro-BFP plasmid	0.75 µg
	EF1α-transposase plasmid	0.37 µg
	Lipofectamine Stem	4 µL

4 Check for transfection efficiency (BFP-labeled cells) on the next day using fluorescence microscopy.

4.1 Passage iPSCs with Accutase to a 10 cm dish when cells are confluent enough for splitting.

> Note
>
> Continue to feed iPSCs daily with Essential 8 media without ROCK inhibitor, and confirm division of stably-expressing transfected cells (should observe local clusters of BFP-fluorescent cells).

5 72:00:00 after transfection, select for transfected iPSCs with [M] 0.5 µg/ml puromycin. 3d

5.1 Confirm purity of surviving transfected cells with fluorescence microscopy. When population is pure, withdraw puromycin.

6 Cryopreserve selected iPSCs with

	A	B
	Essential 8 media	70%
	Knockout serum replacement	20%
	DMSO	10%
	ROCK inhibitor (Supplement)	10 µM

6.1 Proceed to culture and induction to neuronal fate using doxycycline (see “Protocol: iNeuron differentiation from human iPSCs”).